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Contribution of Woodsmoke and Motor Vehicle Emissions to Ambient Aerosol Mutagenicity

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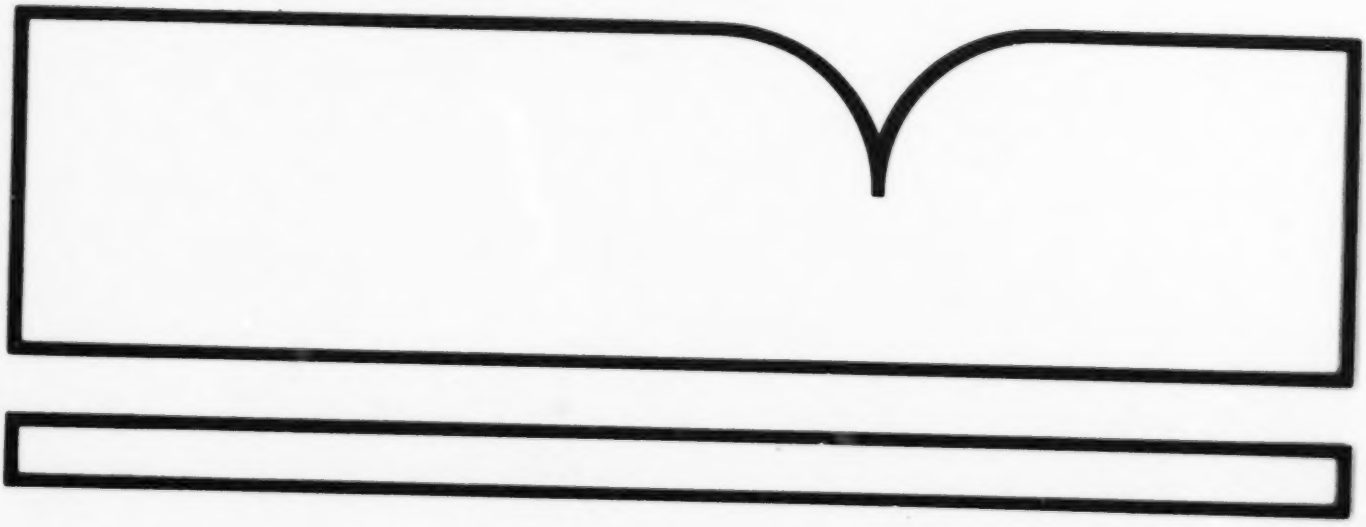

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C. W. Lewis, R. E. Baumgardner, L. D. Claxton, J.Lewtas and R. K. Stevens

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16. ABSTRACT

A receptor model analysis of the mutagenicity of ambient fine particles in air samples collected under EPA's Integrated Air Cancer Project has been performed. The analysis was based on multiple linear regression, using measured concentrations of inorganic chemical elements which are good indicators of likely sources of the mutagenicity. This has allowed the determination of the separate contributions made by woodsmoke and mobile sources to the mutagenicity measured in a residential neighborhood in Albuquerque, NM. During January and February, 1985, these two sources were found to be nearly equal contributors to the average mutagenic concentration (revertants/m^3) of the fine particle ambient aerosol. However, mutagenic potency (revertants /μg extractable organics) of fine particles originating from mobile sources was three times greater than those with a woodsmoke origin. The result is generally consistent with the known potencies of these sources, based on source measurements.

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Contribution of Woodsmoke and Motor Vehicle Emissions to Ambient Aerosol Mutagenicity

Charles W. Lewis,* Ralph E. Baumgardner, and Robert K. Stevens

Atmospheric Sciences Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Larry D. Claxton and Joellen Lewtas

Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

■ A multiple linear regression form of receptor modeling has been used to determine the sources of the mutagenicity (a measure of potential carcinogenicity) of fine-particle ambient aerosol samples collected during the winter in a residential area of Albuquerque, NM. Virtually all the mutagenicity (*Salmonella typhimurium* TA98 +S9) could be accounted for by woodsmoke and motor vehicle emissions. Woodsmoke was found to be the greater contributor to the average ambient concentrations of both extractable organics and mutagenicity. The mutagenic potency (revertants per microgram) of extractable organics traced to motor vehicles, however, was 3 times greater than that with a woodsmoke origin. The results were confirmed by ^{14}C measurements.

Introduction

Mutagenicity is frequently considered as a screening test for the carcinogenicity of compounds to which humans are exposed. Short-term tests for mutagenicity have been used to monitor complex environmental samples for carcinogenic potential (*1*). However, a precise measurement of the contribution of mutagenic sources to total ambient aerosol mutagenicity has not been done. Past estimates of the mutagenicity of ambient aerosol have depended upon source-oriented modeling and estimates of source emissions potencies (the bioassay response per unit mass of extractable organic matter as determined by the slope of a dose–response curve, revertants μg^{-1}) (*2*). In addition to potency estimates, the source-oriented approach also requires knowledge of a source's mass emission rate and an explicit model for the dispersion and possible atmospheric transformation of the mutagens, along with the meteorological and reaction rate data needed for the model's evaluation. In contrast, the direct receptor-oriented approach, demonstrated here, requires only ambient measurements of mutagenicity and of trace elements that are markers for the suspected sources of the mutagens.

The data for this investigation were obtained from field measurements performed during January–February 1985 at Zuni Park, a residential area in Albuquerque, NM, as part of the EPA's Integrated Air Cancer Project (IACP). The site was selected because wintertime organic aerosol concentrations at this site were previously determined to be mostly due to woodsmoke and motor vehicle emissions (*3*). In the present work, woodsmoke was found to be the greater contributor to the average ambient concentrations of both extractable organic matter (EOM) and mutagenicity. The mutagenic potency of the fine-particle EOM traced to motor vehicles, however, was 3 times greater than that with a woodsmoke origin. The ambient measurement derived results are consistent with potencies found from previous source measurements. Finally, ^{14}C measurements were used to confirm the multiple linear regression approach that provided the principal results in this work.

Experimental Section

Sampling. The sampling systems employed at the site were all operated on the same 12-h day (7 a.m. to 7 p.m.) and night schedule. Samples for bioassay were collected on Teflon-coated glass fiber filters (T60A20, Pallflex Corp.) by two Hi-vol samplers equipped with impactors whose cutpoint was 2.5 μm aerodynamic diameter. The aluminum foil impactor surfaces were coated with silicone spray lubricant to minimize particle bounce effects. Two additional samplers of the same type, but employing high-purity quartz microfiber filters (Whatman Ltd.), collected samples for ^{14}C analysis. A dichotomous sampler (Beckman Corp.) employing 2-μm pore-size Teflon filters (Ghia Corp.) collected fine ($\leq$2.5 μm diameter) and coarse (2.5–10 μm diameter) particles for X-ray fluorescence analysis. Samples for total carbon determination were collected with a 22 L min^{-1} 2.5 μm diameter cutpoint cyclone sampler (*4*) employing quartz filters (2500QAST, Pallflex Corp.). Thus, all samplers produced samples which contained the same fine-particle size range, the ambient aerosol size fraction which is both respirable and known to be most strongly associated with mutagenicity.

Sample Analysis. Samples for organic extraction and bioassay were stored for about 6 months at −80 °C from the time of collection until they were extracted. The samples were Soxhlet extracted for 24 h with dichloromethane and the extracts filtered (0.2-μm Millipore-type FG filters). Depending on availability and atmospheric mass loading, one or both of the duplicates from each sampling period were used in this step. The extracts were concentrated on a rotary evaporator with a 35 °C water bath to about 4 mL and quantitatively transferred to 10-mL volumetric flasks. Portions of the extract not immediately used for preparation of gravimetric or bioassay samples were stored at −20 °C.

A gravimetric determination of the EOM in each extract was performed by placing duplicate 0.2-mL aliquots in tared aluminum weighing pans and allowing the solvent to evaporate in a fume hood, followed by desiccation for 24 h before reuse. Duplicates which failed to agree within 5% were repeated with additional portions of the extract.

The portion of each extract available for bioassay generally had an EOM content exceeding 12 mg. Each portion was solvent exchanged into dimethyl sulfoxide and assayed by previously published procedures (*5*, *6*) by using *Salmonella typhimurium* TA98 both with and without Aroclor induced CD-1 rat liver homogenate (+S9 and −S9, respectively). The "standard" S9 concentration used was verified to be within the optimum range. Nearly every sample was significantly more mutagenic with S9 than without. Only the +S9 results are given here, with those for −S9 to be presented elsewhere. The uncertainty of the +S9 bioassay measurements, based on one-half the difference between duplicate determinations performed on every sample, averaged 14%.

Dichotomous sampler Teflon filters were analyzed by an X-ray fluorescence procedure (*7*) to determine concentrations of a range of some 20 elements including Pb, Br, K, and Fe. The average uncertainties of the latter four concentrations ranged from 9 to 13%.

Quartz filters from the cyclone sampler were analyzed for concentrations of volatilizable and elemental carbon by using a two-step thermal method described previously (*4*). The average uncertainty in the determination of total (volatilizable and elemental) carbon concentrations was 14% from three replicated measurements on each of 15 samples.

A small number of quartz Hi-vol filters were measured for ^{14}C content by the National Bureau of Standards (NBS) with both low-level counting and accelerator mass spectrometric procedures.

Source Apportionment Results

The single-tracer multiple linear regression (MLR) method (*8*) of receptor modeling assumes that the ambient concentration of a pollutant can be represented as a sum of terms: each expressing the contribution of a particular source category, and each being the product of the concentration of a chemical species uniquely associated with that source times an unknown coefficient to be determined by regression. Many studies have demonstrated the usefulness of Pb as a tracer of motor vehicle emissions, including both leaded and unleaded fuel vehicles (*9*). Our fine-particle ambient measurements showed a very strong Pb–Br correlation coefficient (0.99) and an average Br/Pb ratio (0.33 ± 0.05) close to that found in fresh automotive emissions, giving strong support to this use of Pb in the present study.

For a tracer of woodburning emissions, a soil-corrected potassium quantity similar to that discussed and employed in previous studies (*3*, *8*) was used:

$$[K'] = [K] - 0.45[Fe] \quad (1)$$

Here [K] and [Fe] are ambient fine-particle potassium and iron concentrations ($\mu g\ m^{-3}$) measured during the same sampling period, and 0.45 is the best estimate of the K/Fe ratio in local soil. The latter value was obtained from the average K/Fe ratio found for the ambient *coarse*-particle samples, in which the correlation of these elements was 0.98 over 103 samples. (In the fine-particle samples the correlation coefficient of [K′] and [Fe] was only 0.21, further emphasizing their different origins in the fine fraction.) Overall the average soil correction was 20%, but for daytime samples it increased to 50%. The resulting values of [K′] showed very strong nighttime maxima, consistent with a typical residential woodburning pattern.

Unquestionably the potassium fraction of woodsmoke varies greatly with fuel type and burn conditions. This variability can be a serious problem in a chemical mass balance approach to source apportionment, which requires the average potassium fraction to be estimated from at best a limited number of source sampling measurements. It is the significant advantage of the MLR approach that the emission variability is dealt with in a way that avoids such a heavy reliance on choosing the correct source signature.

Other work has suggested methyl chloride (*10*) and the polycyclic aromatic hydrocarbon retene (*11*) as possible woodsmoke tracers. For methyl chloride a large seasonally variable background poses a severe challenge to the precision of this application. Thus, even under conditions of strong (>50 $\mu g\ m^{-3}$) woodsmoke impact, ambient and background methyl chloride concentrations must both be known to a few percent to allow estimating the woodsmoke impact to an accuracy of no better than 20–40% (*10*, *12*).

Table I. Average Ambient Concentrations and *S. typhimurium* TA98 (+S9) Mutagenic Potencies Attributed to Woodsmoke and Motor Vehicles in Albuquerque during January–February 1985

	wood-smoke	motor vehicles	other	total
extractable organics, $\mu g\ m^{-3}$	14.8 ± 0.6	3.0 ± 0.9	1.1 ± 0.9	18.9
mutagenicity, revertants m^{-3}	18.8 ± 2.0	11.0 ± 3.0	2.5 ± 3.1	32.2
mutagenic potency, revertants $(\mu g\ organics)^{-1}$	1.3 ± 0.2	3.7 ± 1.5		

Expressed another way the signal to background ratio for methyl chloride is at least an order of magnitude smaller than for soil-corrected potassium. Methyl chloride has the further uncertainty of being a gaseous tracer for a fine-particle impact.

Retene appears to be a more specific tracer, found in emissions from softwood combustion but not hardwood (*11*). Since the dominant wood burned in Albuquerque is a softwood, pinion pine, retene was an interesting possibility. However the much greater analytical costs associated with retene, compared with potassium, discouraged its consideration.

Organics. Equation 2 is the MLR result for the measured ambient concentration of extractable organic matter EOM_i ($\mu g\ m^{-3}$) for observation i, determined from 44 (18 day, 26 night) samples with $\mu g\ m^{-3}$ as the units of the tracer

$$[EOM_i] = (204 \pm 8)[K_i'] + (10.6 \pm 3.0)[Pb_i] + 1.0 \pm 0.9 \quad (2)$$

$$r = 0.98$$

concentrations $[Pb_i]$ and $[K_i']$. The uncertainties are in terms of standard errors.

Considerable effort was spent in assessing the stability and accuracy of eq 2. Forty-seven complete cases were originally available, but three were omitted which had very large values of the dependent variable (EOM), far from the approximately normal distribution of the remaining cases. Omitting the three cases had little effect on the regression coefficients (except for reducing their standard errors) but caused the correlation coefficient between [Pb] and [K′] to decrease from 0.78 to 0.46, a more satisfactory situation for nominally independent variables. Omitting as many as 14 additional cases caused a further decrease to 0.13, with negligible change in the regression coefficients. A jackknife procedure (*8*, *13*) was also applied to obtain nonparametric estimates (results that are less dependent on the stringent assumptions that underlie classical regression) of the regression coefficients and their standard errors. For the original data set the jackknife estimates differed in some details from those in eq 2, while for the 44 case set they were virtually the same.

The average source apportionment of EOM shown in the first row of Table I was constructed from eq 2 and the average of each tracer concentration over the 44 samples. The woodsmoke contribution to EOM is dominant, partly because there were more night than day samples.

Mutagenicity. Equation 3 gives the MLR result for the ambient concentration of mutagenicity R_i (revertants m^{-3}) for the same 44 cases on which eq 2 is based. The

$$[R_i] = (258 \pm 27)[K_i'] + (38.6 \pm 10.4)[Pb_i] + 2.5 \pm 3.1 \quad (3)$$

$$r = 0.90$$

multiple correlation coefficient associated with eq 3, while

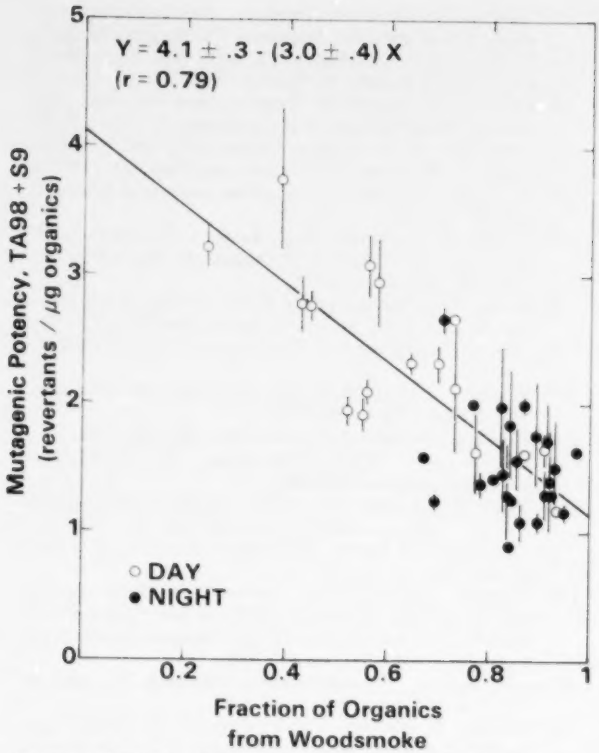

Figure 1. Mutagenic potency (*S. typhimurium* TA98 with S9 exogenous activation) of dichloromethane-extractable organics in ambient fine-particle samples versus fraction of extractable organics due to woodsmoke. Error bars are equal to half the difference in duplicate bioassay results.

satisfactory, is smaller than that for eq 2. This is surely due in part to the poorer measurement accuracy of R_i compared with EOM_i. The jackknife estimates of the regression coefficients and their standard errors were essentially unchanged from those in eq 3.

The average source apportionment of mutagenicity, using eq 3, is shown in the second row of Table I.

Potencies. While the contributions of particular sources to ambient concentrations of organics and mutagenicity will naturally be highly dependent on the sampling site and period, the potencies of specific source emissions should have a more universal significance. The *ambient measurement derived* potencies of woodsmoke and motor vehicle emissions are given in the third row of Table I, obtained from the ratio of the preceding entries in each column.

Since Table I shows that the contributions to both extracted organics and mutagenicity from "other" sources are insignificant, the woodsmoke and motor vehicle potency values may also be obtained graphically. Figure 1 shows the bulk potency of each ambient fine-particle sample plotted against the fraction of extractable organics in the sample due to woodsmoke, using eq 2. Qualitatively, the negative slope of the linear least-squares fit to the data shows that the potency of ambient woodsmoke is less than that of motor vehicle emissions. Quantitatively, the ordinates at the 0 and 100% abscissa values agree well with the corresponding potencies given in Table I.

Figure 1 emphasizes the dominant contribution that woodsmoke makes to organics concentrations at night. It is important to note, however, that apart from this source strength influence there seems to be no other diurnal effect on mutagenicity. That is, the linear relationship appears to fit day and night observations equally well.

Table II. Comparison of ^{14}C and MLR Estimates for Percentage of Fine-Particle Carbon from Woodburning for Six Albuquerque Samples

date	sampling period	% fine-particle C from woodburning based on ^{14}C measurements	% fine-particle C from woodburning derived from MLR model
1-13-85	night[a]	75 ± 6	69 ± 10
1-18-85	night	66 ± 3	75 ± 11
1-24-85	day[b]	58 ± 6	52 ± 12
2-02-85	night	80 ± 5	98 ± 11
2-04-85	day	61 ± 12	49 ± 13
2-16-85	night	66 ± 3	61 ± 10
av		68	67

[a] 7 p.m. to 7 a.m. [b] 7 a.m. to 7 p.m.

Validation by ^{14}C. To provide an independent check of the MLR results, the National Bureau of Standards analyzed several samples for ^{14}C content. These samples were collected on quartz filters and were not extracted. The ^{14}C approach (*14*) provides a direct (nonstatistical) estimate of the fraction of aerosol carbon that originates from woodburning (i.e., carbon from fossil fuels is devoid of ^{14}C). Table II shows a comparison of the ^{14}C and MLR results. The latter were based on the regression equation (44 cases)

$$[C_i] = (190 \pm 9)[K_i'] + (14.1 \pm 3.7)[Pb_i] + 1.4 \pm 1.1 \quad (4)$$

$$r = 0.97$$

analogous to eq 2 but for total fine-particle carbon instead of extractable organics, to be consistent with the ^{14}C analyses. Thus the woodburning percentage values from MLR shown in Table II are simply the ratios of 190 $[K_i']$ to $[C_i]$.

The ^{14}C percentages shown are the NBS-measured fractions of "contemporary" carbon in each sample divided by the correction factor 1.10, which relates to the average age of the wood (*15*), estimated to be 50 years. For wood harvested in 1985, the maximum possible correction factor is 1.15, which would apply to 31-year-old wood (16).

In virtually every case the ^{14}C and MLR results agree within their uncertainty limits. Overall the agreement supports the validity of the statistical model and tracer quantities from which the principal results of this work (Table I) were derived.

Discussion

Since the mutagenic potencies given in Table I are based solely on ambient measurements, they implicitly include changes in potency, if any, resulting from atmospheric transformations of the original source emissions. This is desirable if the values are to be used in estimating human exposure. Depending on the conditions (irradiation, concentrations of reactants, temperature, etc.) smog chamber studies have shown that the mutagenic potency of woodsmoke can be increased (*17*), decreased (*18*), or left unchanged (*19*), with a similar situation for motor vehicle emissions (*20*). Thus, it is difficult to anticipate the extent to which ambient-based measurements of potencies will reflect the values found from source emissions testing. The following discussion demonstrates there is a discernible consistency between the potencies given in Table I and previous measurements of potencies from source emissions testing. However, the variability in the source results and lack of source results specifically from the Albuquerque airshed do not allow detailed conclusions to be drawn

concerning the role of atmospheric transformation on mutagenicity in this airshed.

We first consider the available data from mutagenic source testing on woodsmoke emissions. Using *S. typhimurium* TA98 with S9, particles collected by dilution sampling from an airtight woodstove gave potencies of 1.3 and 0.9 revertants μg^{-1} for softwood (pine) and hardwood (oak) fuels, respectively (*21*). The source value for pine is identical with the value shown in Table I for ambient woodsmoke in Albuquerque, where the common wood burned is pinion pine. Overall, studies of woodsmoke emission sources in the U.S. have reported (*17, 21*) values ranging from 0.12 to 1.3 revertants μg^{-1}. Scandinavian studies, which use different source sampling methods and conditions (*22, 23*), have reported somewhat higher potencies for emissions from Swedish (0.2–2.8 revertants μg^{-1}) and Norwegian (4.5 revertants μg^{-1}) woodstoves.

Table I shows the ambient-derived potency of aerosol organics originating from motor vehicles to be 3 times greater than that with a woodsmoke origin. Correspondingly, emission studies have consistently reported the potencies of particle EOM from mobile source dilution sampling experiments to be higher than those from woodstoves. In the mobile source studies significant differences are observed between the organics from different vehicle classes (e.g., diesel autombiles and trucks, catalyst and noncatalyst gasoline automobiles). The mutagenic potency of emissions from diesel cars (*24–26*) ranges from 3 to 16 revertants μg^{-1}, for noncatalyst gasoline cars (*24–26*) burning leaded gasoline from 9 to 16 revertants μg^{-1}, and for catalyst cars (*26*) burning unleaded gasoline from 10 to 23 revertants μg^{-1}. A few cars were reported to be as low as 1 revertant μg^{-1}. Only particles from heavy-duty diesel trucks and buses have consistently been reported to have potencies in the range of 1 revertant μg^{-1} and less (*26, 27*). While it is not possible, at this time, to apportion the mutagenicity from mobile sources in the Albuquerque airshed between different types of vehicles, the average value of 3.7 revertants μg^{-1} (Table I) derived from the ambient measurements appears consistent with the previous source studies and the motor vehicle mix in Albuquerque.

An important feature of the receptor-oriented approach is that weighted averages, over the thousands of individual sources and their myriad of emission rates, are implicitly and automatically performed for the two source classes considered without detailed knowledge of the emission rates or populations. The implicit inclusion of the effects of atmospheric transformation is the other distinguishing feature of the receptor approach.

A similar application of this statistical method of mutagenic apportionment has been performed recently (*28*) but in a more complex airshed and without benefit of an independent nonstatistical check such as was provided by the ^{14}C comparison.

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